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Gametogenesis and Early Development of the Temperate Coral 'Astrangia danae' (Anthozoa: Scleractinia)

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16. ABSTRACT
The growing interest in coral reefs and coral biology has not led to many studies of reproduction. Recent reviews of coelenterate reproduction and development (Cambell, 1974; Connell, 1973; Mergner, 1971; Miller and Wyttenbach, 1974; and Uchida and Yamada, 1968) have dealt mainly with Scyphozoa and Hydrozoa, and other anthozoan groups such as the anemones. While it seems likely that patterns of reproduction in corals may be similar to those in anemones, we lack detailed studies of the corals themselves. Our present knowledge is largely based on the studies during the Great Barrier Reef Expedition by Marshall and Stephenson (1933), who found sporadic occurrences of gonadal tissues and reproductive products in most of the species examined. For some species they were only able to locate individuals of one sex. Several species of Pocillopora were shown to brood their embryos up to the planula stage, but early stages of embryogenesis were not observed. Subsequent studies on coral reproduction have been limited to observing the release of planulae (Stimson, 1976, 1978; Harrigan, 1972) or the development, settlement and metamorphosis of planulae after release by the parent colony (Abe, 1937; Atoda, 1947, 1951; Lewis, 1974). Recent studies by Rinkevich and Loya (1979, a, b) on reproduction of the Red Sea coral Stylophora pistillata include some histological information on gonadogenesis but emphasize the ecological aspects of reproduction in this species. They do not report observing the transition from ovum to planula. No systematic studies have described gametogenesis, spawning, fertilization and early embryogenesis in any coral species.

This report presents the results of an 18-month study of the reproductive activities of the temperate scleractinian coral Astrangia danae living near the northern limit of its geographic distribution. Along the southern end of Narragansett Bay, Rhode Island, A. danae occurs as small encrusting colonies on rocky surfaces and shells (Agassiz and Fewkes, 1889). It is found in nature both with and without the endosymbiotic dinoflagellates known as zooxanthellae and is remarkable among corals in tolerating an annual temperature range of -2°C to 22°C.

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GAMETOGENESIS AND EARLY DEVELOPMENT OF THE TEMPERATE CORAL *ASTRANGIA DANAE* (ANTHOZOA: SCLERACTINIA)

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The growing interest in coral reefs and coral biology has not led to many studies of reproduction. Recent reviews of coelenterate reproduction and development (Campbell, 1974; Connell, 1973; Mergner, 1971; Miller and Wyttenbach, 1974; and Uchida and Yamada, 1968) have dealt mainly with Scyphozoa and Hydrozoa, and other anthozoan groups such as the anemones. While it seems likely that patterns of reproduction in corals may be similar to those in anemones, we lack detailed studies of the corals themselves. Our present knowledge is largely based on the studies during the Great Barrier Reef Expedition by Marshall and Stephenson (1933), who found sporadic occurrences of gonadal tissues and reproductive products in most of the species examined. For some species they were only able to locate individuals of one sex. Several species of *Pocillopora* were shown to brood their embryos up to the planula stage, but early stages of embryogenesis were not observed. Subsequent studies on coral reproduction have been limited to observing the release of planulae (Stimson, 1976, 1978; Harrigan, 1972) or the development, settlement and metamorphosis of planulae after release by the parent colony (Abe, 1937; Atoda, 1947, 1951; Lewis, 1974). Recent studies by Rinkevich and Loya (1979, a, b) on reproduction of the Red Sea coral *Stylophora pistillata* include some histological information on gonadogenesis but emphasize the ecological aspects of reproduction in this species. They do not report observing the transition from ovum to planula. No systematic studies have described gametogenesis, spawning, fertilization and early embryogenesis in any coral species.

This report presents the results of an 18-month study of the reproductive activities of the temperate scleractinian coral *Astrangia danae* living near the northern limit of its geographic distribution. Along the southern end of Narragansett Bay, Rhode Island, *A. danae* occurs as small encrusting colonies on rocky surfaces and shells (Agassiz and Fewkes, 1889). It is found in nature both with and without the endosymbiotic dinoflagellates known as zooxanthellae and is remarkable among corals in tolerating an annual temperature range of −2° C to 22° C.

Materials and Methods

Collection

Colonies of *A. danae* were collected by SCUBA divers using chisels and knives to remove the corals from their rocky substrate. They were placed in plastic

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TABLE I

Criteria for differentiating stages of gonadal development in Astrangia danae, *as seen in histological sections of the mesenteries.*

Stage	Male	Female
0	No spermaries in mesentery; completely dispersed spermatocytes	Degenerate ova
I	Small clusters of interstitial cells in mesoglea of mesentery	Enlarged interstitial cells with large nuclei in mesoglea of mesentery
II	Spermary boundaries distinct but spermatocytes with small nuclei	Accumulation of small amount of cytoplasm around nuclei
III	Spermatocytes larger with large nuclei	Increasing amount of cytoplasm around nuclei but no vitelline membrane
IV	Spermatocytes undergoing meiosis and nuclear condensation but tails not evident	Oocytes full size with vitelline membrane; chromatin dispersed
V	Spermatozoa with tails; ready to spawn	As in stage IV but chromatin condensed; seldom seen
S	Spawned spermary	—

bags containing sea water and returned to running seawater aquaria where they were maintained at close to the temperature of the bay. Specimens used for histological examination were fixed within 36 hr of their return to the laboratory.

Histological preparation

Several different fixatives were tried, including Bouin's and Fleming's (Gray 1954) and a modified Helly's (Yevich and Barszcz, 1977). The best results were obtained by overnight fixation with Helly's fixative; it was more effective in retaining all of the many types of intracellular granules found in the gastrodermal tissues. Primary fixation with Helly's for 2 hr followed by post-fixation with a mixture (1:1) of Helly's and 2% OsO_4 was also used during the latter part of the study and was superior for fixing lipid reserves and oocyte yolk deposits.

After fixation, the corals were decalcified for 24 hr in Decal (Harleco Co.) and rinsed for 24 hr in running tap water. Dehydration, clearing and paraffin infiltration were done with a Technicon Tissue processor using Technicon reagents S-29 and UC-670 for dehydration and clearing and Paraplast for infiltration and embedding. Initial problems with tissue cracking were solved by reducing the three infiltration steps from 30 min to 15 min each.

Tissues were step-serially sectioned, 4–7-μm thick, and stained with Harris' hematoxylin and eosin (H & E) or Heidenhain's aniline-blue (Luna, 1968).

Quantification of gonad stages

A system of six spermary stages and five oocyte stages (Table I) was used to quantify the histological observations of gonad developmental stages. Five mesenteries were selected from each colony sectioned and the number of each stage of spermary or oocyte was counted. These data will be included in the doctoral dissertation of A.S.F. (University of Rhode Island, expected date 1980). The percent occurrence of a given stage was then calculated for each sex for each sampling date (Fig. 1). An example of typical data and of the method used in calculating the results is presented in Table II.

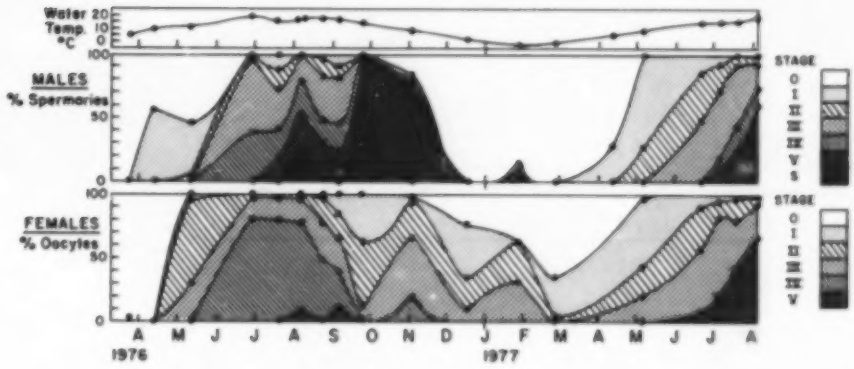


FIGURE 1. Temporal changes in the development of the gonads of *Astrangia danae* in Narragansett Bay. The stages (defined in Table I) were calculated from counts made on histological sections of the mesenteries. Temperatures reported were taken at the collection sites.

Spawning and fertilization

Live coral colonies were sexed by examining reproductive products in freshly collected bits of gonadal tissue. Fine-tipped forceps were inserted through the mouths of individual polyps and small pieces of mesenterial tissue removed for examination under a compound microscope.

Sexed colonies were placed in individual finger bowls filled with filtered sea water. Spawning was induced by placing small pieces of male gonadal tissue inside female polyps and female tissue inside male polyps. Fertilization of the eggs was accomplished by pipetting the freshly spawned eggs into finger bowls and adding water from the bowls of spawned males. Water in the bowls with developing coral larvae was changed at least twice a day during the first 48 hr and once a day thereafter. Samples of the sperm, unfertilized and fertilized eggs, and various stages of embryonic development were observed and photographed with a Zeiss photomicroscope.

Additional samples of the various embryonic stages were fixed in sea water-glutaraldehyde (3%), post-fixed in OsO_4 (1% in sea water) and embedded in Spurr's medium (Polysciences, Inc., Warrington, Pa.). Silver sections were stained with 2% uranyl acetate and Reynold's lead citrate, and observed with a Zeiss electron microscope.

RESULTS

General

As in other anthozoans, the gonads of *A. danae* are located in the mesenteries, between the mesenterial filaments and the muscle bands (Fig. 2). Each polyp had up to 24 mesenteries, and all were capable of bearing gonads.

Colonies of different sizes were used in this study. The smallest ones collected, of from 6 to 9 polyps (estimated age of 1 to 3 years, based on growth rates in the laboratory), were already capable of sexual reproduction.

TABLE II

Gonad-stage data for colonies collected on June 20, 1977: an example of typical data and of the method used to calculate the average percent occurrence of each stage seen in Figure 1.

Colony No.	Sex	Mesentery Number																			
		1				2				3				4				5			
		Stage				Stage				Stage				Stage				Stage			
		I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
45	M	4		2		3				9	3			4	3			1	6		
46	M	5	5	12		2	5	4			14	20		3	8	2		6	24		
47	F		17			11	18			12	17				4			4	7	6	
48	M		1	1				3													
49	M		2	1			4	4		1	1	3			3	1			2		
50	F		5	21	4			8			18	20			3	22	5		6	29	
51	M	3	5	4			6	1			2	14		1	4	9		2	1	3	
52	F	1	6	11			3	9	4			28	8	1	6	9	3	2	4	9	6
53	M	2	4	29			4	12	3		11	10		1	2	11		3	10	23	
54	F	2	5	6	2	3	5	7			2	7			7	7	1	1	5	11	3
55	F	3	6	7		2	2	3	2		2	5	1	3	2	13				7	
56	F		7	1		3	5	2	1		1		4	1	9	13		7	3	7	
57	F		6	7																	

	Total staged	Average percent each stage on June 20, 1977						
		0	I	II	III	IV	V	S
Oocytes	556	0	10	33	49	8	0	—
Spermaries	352	0	14	37	48	1	0	0

All polyps belonging to a given colony were of the same sex; only one hermaphroditic polyp was found out of about 5000 polyps observed. Of the 153 colonies sectioned for this study, 3 had no recognizable gonadal material in the sections examined. Of the remaining colonies, 77 were male and 73 female, indicating a 1:1 sex ratio for this species.

An egg production estimate of 6000 eggs per polyp was obtained by counting the number of eggs present in vertical and cross sections of several polyps from a single colony. The polyps of this particular colony seemed to have a higher than usual egg density; the average egg density for this species is probably closer to two-thirds to one-half of this value.

Seasonal trends

Many stages of gonad development were simultaneously present in mesenteries of the same polyp, and such differences could also be seen between polyps in one colony and between colonies collected on the same date (Table II). Nevertheless, when the average percent occurrence of each stage is plotted (Fig. 1) clear seasonal trends are apparent.

Gametogenesis started in the early spring (March–April) and proceeded through June and July. The spawning season was from early August through September, when water temperatures were at their peak. In September 1976 a second cycle of gametogenesis started, but no evidence of a second spawning episode was encountered. Finally, the remaining unspawned gametes gradually disappeared over the winter months.

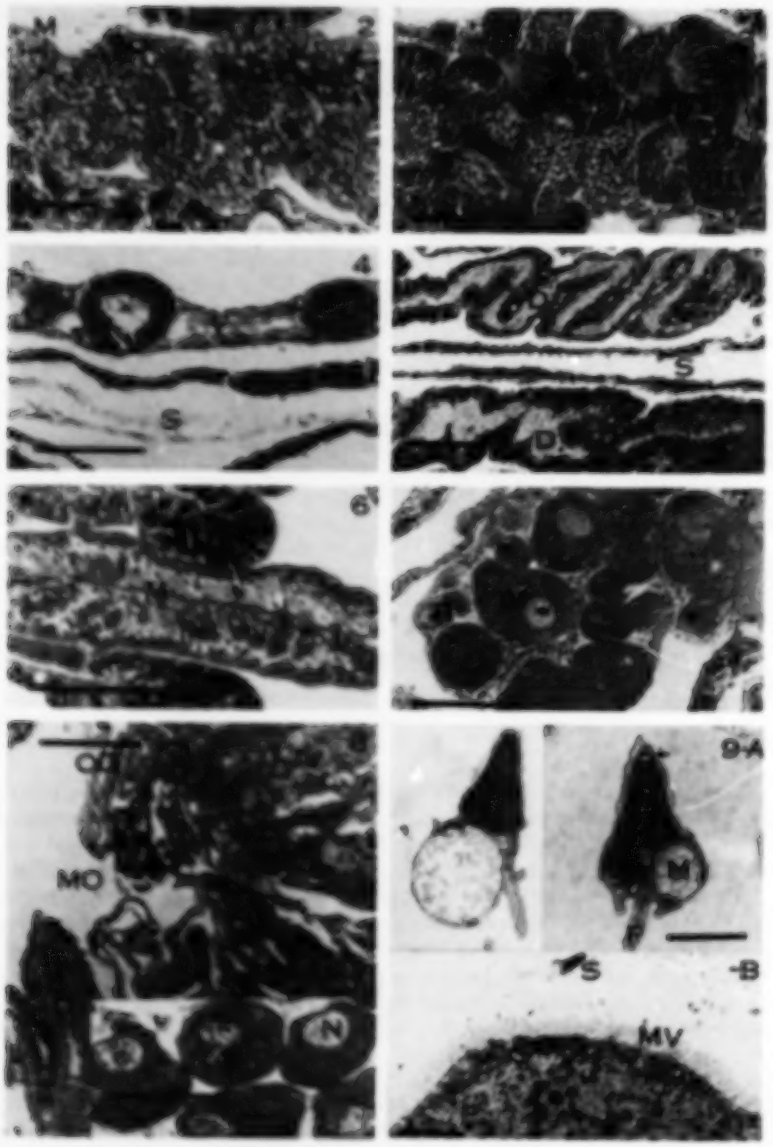
Separate descriptions of the gametogenic cycles of male and female colonies follow.

Spermatogenesis

It is difficult at this level of observation to distinguish between primordial germ cells and spermatogonia, and the transition of the latter into spermatocytes (Giese and Pearse, 1974). Therefore, the term spermatocyte was used to identify all stages of differentiation before the appearance of the spermatozoan tails.

Spermary formation began in the early spring with the appearance of interstitial cells (Hyman, 1940; Campbell, 1974) in the mesoglea. Here the interstitial cells formed small membrane-bound aggregates (stage I) (Fig. 2). The number and size of the cells in these early spermaries gradually increased, but the spermatocytes still retained an appearance similar to that of the initial interstitial cells (stage II). The spermatocytes then changed in staining characteristics, developing a greater affinity for hematoxylin, an indication of increased nucleic acid content (stage III). The spermatocytes became much more numerous and smaller, with condensed nuclei; however, spermatozoan tails could not be distinguished in the preparations (stage IV) (Fig. 3). Finally long tails became evident, and the spermatozoa appeared ready to be spawned (stage V) (Fig. 3).

Spawning resulted in the appearance of empty pockets in the male gonads where the spermaries had once been (stage 5) (Fig. 4). The major period of spawning during August and early September 1976 was followed by the reappearance of large numbers of early stage spermaries (I and II) in the mesenteries. By November late stage spermaries (III–V) were seen in many polyps, but there was no evidence that the spermatozoa were being released. Instead, by December



FIGURES 2-8. Photomicrographs of histological preparations of tissues of *A. dianae* illustrating the gametogenic sequences described in text. Tissues were fixed in Helly's fixative and stained with Heidenhain's aniline-blue. Abbreviations used: D—dispersed spermatocytes; E—endoderm; F—mesenterial filament; I—stage I; II—stage II; III—stage III; IV—stage IV; M—mesoglea; MO—mouth; N-nucleus OD-oral disk; R—partially resorbed oocyte; S—septal space; T-tentacle; V-stage V; X—spawned spermary.

FIGURE 2. Cross-section of gonad-bearing portion of a mesentery with spermaries in developmental stages I and II. Scale bar equals 50 μm.

or January the gonads typically looked like the one shown in Figure 5. The membranes surrounding the spermaries and the tails of the spermatozoa were no longer visible. Lesser numbers of spermatocytes were observed in later collections, so it appeared that these cells were being absorbed.

Oogenesis

The early detection of gametogenesis in the mesenteries of female colonies was difficult because it involved the recognition of single interstitial cells, rather than the aggregates of cells seen in male gonads. This may account for the apparently later initiation of gametogenesis by female polyps then by male polyps (Fig. 1).

Stage I oogonia were found along the mesogleal lamella, where they could be distinguished by their large nuclei and nucleoli (Fig. 6). The smallest oogonia seen were about 5 μm in diameter. Very little cytoplasmic material could be seen around the nuclei at this stage. Vitellogenesis started after the oogonia entered the mesoglea (stage II). As the oocytes continued to grow, both the cytoplasm and the nuclei increased in size (stage III). Oocytes were assigned to the stage III growth phase until they reached nearly their full size. The increase in size is dramatic: there is a 600-fold increase in volume. The source material for this increase is not known, but it appears that some smaller oocytes may have been resorbed as the larger oocytes grew (Fig. 7). The formation of the vitelline membrane, which consists of a layer of cortical vesicles just beneath the cell membrane, marked the attainment of stage IV (Fig. 7). The vesicles made the peripheries of the cells stain dark blue with both the H and E and the Heidenhain's stains. The now mature eggs apparently remained in a stage IV condition until spawned sometime in August and early September.

In a small fraction of observations, a situation described as stage V was seen. The nuclear material appeared condensed rather than dispersed (Fig. 8). This stage may represent part of a final meiotic reduction; however, the release of polar bodies was not observed. The low frequency with which this stage was encountered suggests that it lasted only a short time before the eggs were spawned. After spawning the remaining mesenterial tissues were very broken up and disorganized.

FIGURE 3. Oblique section through a mature male gonad with spermaries at various stages of development up to stage V. Scale bar equals 100 μm.

FIGURE 4. Longitudinal section of a male gonad containing partially and completely spawned spermaries (stage S). Scale bar equals 100 μm.

FIGURE 5. Longitudinal section of several male mesenteries showing absence of spermary membranes and dispersed, tail-less spermatocytes. Scale bar equals 200 μm.

FIGURE 6. Early oogenesis seen in a longitudinal section of a mesentery. Stage I and II oocytes are strung along the mesogleal lamella. Scale bar equals 100 μm.

FIGURE 7. Section through ovary showing mature oocytes with well developed vitelline membrane (stage IV). Notice that some of the smaller oocytes seem to be partially resorbed. Scale bar equals 100 μm.

FIGURE 8. Longitudinal section of a female polyp fixed while spawning. Compare the nuclear material of the spawned ova (insert) with that of the oocytes in Figure 7. Upper scale bar equals 500 μm; insert scale bar equals 100 μm.

FIGURE 9. Electron-micrographs of *A. danae* egg and spermatozoa. A. Longitudinal sections through spermatozoa show single mitochondrion and large lipid body. Notice less dense material anterior to the tip of the nucleus (arrow). Scale bar equals 1 μm. B. Cross section of outer portion of an unfertilized spawned egg. A layer of sub-cortical vesicles lies beneath the microvillous oolema. Yolk granules and mitochondria are abundant within the cell. Scale bar equals 5 μm. Abbreviations: CG—cortical granules; F—flagella; L—lipid body; M—mitochondrion; MV—microvilli; N—nucleus; S—spermatozoon; Y—yolk granule.

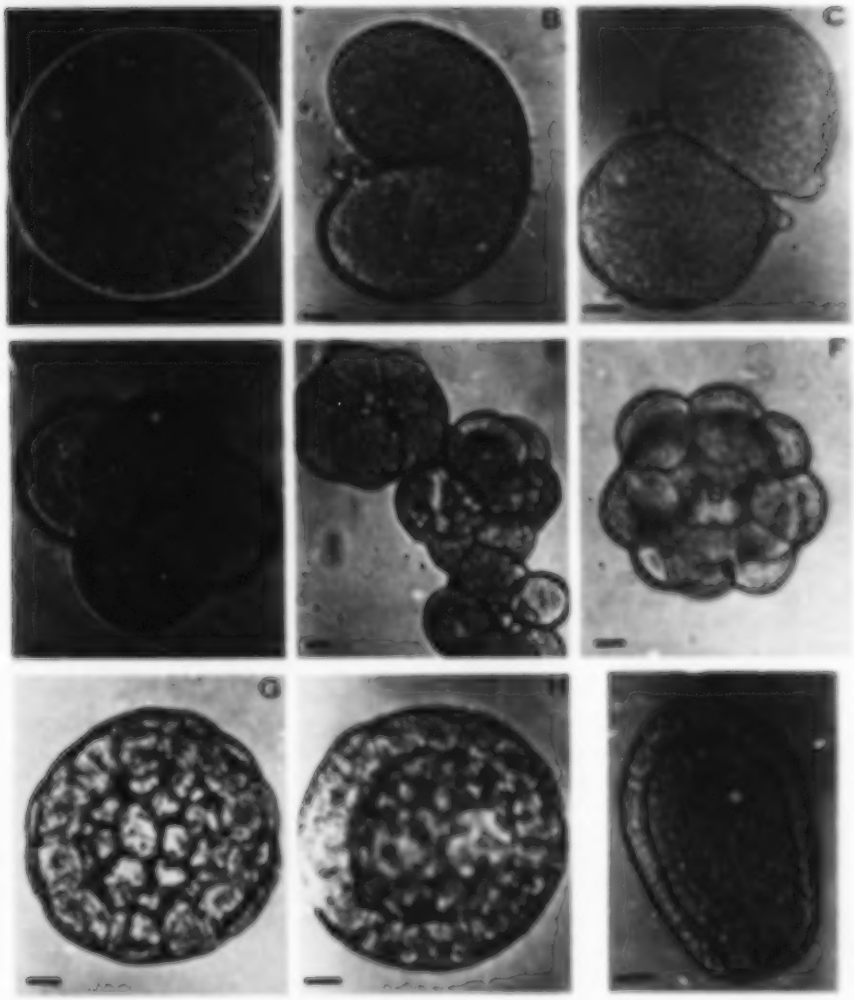


FIGURE 10. Photomicrographs of various stages in the embryonic development of *Astrangia danae*. A. Unfertilized egg with eccentric nucleus. B. First cleavage proceeds from animal to vegetal pole. C. First division complete; notice small protuberances being pinched off at vegetal pole. D. Pseudospiral arrangement of blastomeres after second division. E. Irregularly shaped embryos resulting from nonsynchronous third and fourth divisions. F. Embryo of around 20 blastomeres, with large blastocoele. G. Sixty-four cell stage; blastomeres still rounded. H. Blastula stage; blastomeres compressed. I. Planula stage, showing apical tuft of cilia and oral pore. Abbreviations: AP—animal pole; AT—apical ciliary tuft; B—blastocoele; N—nucleus; OP—oral pore. Scale bars are 10 μm.

Few eggs remained in the gonads. By mid-September, however, stage I and II oocytes began to appear in the mesenteries again. Although some of these oocytes developed as far as stage IV, they were never as numerous and they never attained the size of the mid-summer oocytes. As with the male colonies, there was no evi-

dence of a second spawning cycle. By December signs of oocyte absorption began to appear, such as the disappearance of yolk material. The process of absorption continued throughout the winter months until the unspawned oocytes were almost completely absorbed.

Spawning

Spawning was first observed in the laboratory when several colonies were inadvertently subjected to heat and possibly low-oxygen stress. It was again accidentally induced during the course of another experiment, possibly by low oxygen or a combination of unknown stress factors. Spawning was later experimentally induced, as previously described, by stimulating polyps of one sex with bits of gonadial tissue of the other sex. In all cases, a certain behavior pattern was noticed. The polyps first expanded to an extreme degree, but the tentacles remained short and stubby, very unlike the appearance of a polyp expanded in a feeding posture. The oral disk was very prominent, with the mouth tightly closed and puckered at the center. At the moment of spawning the polyp would suddenly shorten its column and a narrow stream of eggs or sperm would shoot out of its mouth. Within a few minutes the polyp would be fully expanded again, and the contraction with gamete ejection quickly repeated. Individual polyps were seen to go through this cycle up to six or seven times.

In most cases one polyp of a particular colony went through a couple of ejection cycles before any of the other polyps joined in. Not all polyps of a spawning colony spawned, and some seemed to be spawning more eggs or sperm than others.

The mature spermatozoa were around 2–3 μm in length, not including the tail. They were characterized by a single large mitochondrion and a large, electron-dense lipid body located at the base of the conically elongated nucleus (Fig. 9A). No acrosome was seen but, as can be seen in Figure 9A, there was an accumulation of moderately dense material anterior to the tip of the nucleus, similar to that reported by Hinsch and Clark (1973) for *Astrolis*.

The mature ova were around 100 to 130 μm in diameter, with an asymmetrically located nucleus (Fig. 10A). The ooplasm was densely populated with yolk bodies and mitochondria. The surface of the cell was microvillous, with a subsurface layer of cortical granules (Fig. 9B).

Fertilization and development

Most of the spawned eggs quickly settled to the bottom of the culture dish, where they could be pipetted and concentrated before fertilization. Figure 10 presents a sequence of photomicrographs of progressive stages of development of *A. danae* embryos. No fertilization membrane could be distinguished by light microscopy. The first division took place within the first hour after fertilization (AF). The cleavage furrow started at the animal pole (near the asymmetrically located nucleus) and progressed inward towards the vegetal pole (Fig. 10B). An event frequently seen at this time was the pinching off of two small portions of cytoplasmic material at the vegetal end (Fig. 10C). We have no explanation of this phenomenon. The timing of subsequent divisions varied between batches of eggs obtained on different occasions, but they were generally 30 to 60 min apart. The second division was always synchronous between the two blastomeres. The cleavage plane was radial but the blastomeres rearranged themselves after the division into a pseudospiral (Mergner, 1971) formation (Fig. 10D). Subsequent

divisions were not always synchronous, resulting in a variety of embryos of different cell numbers and shapes (Fig. 10E). A blastocoele formed early in the cleavage sequence (Fig. 10F); blastocoeles were observed in embryos with as few as 12 blastomeres. At the *ca.* 64-cell stage, the blastomeres were still fairly rounded, especially on their inner surface (Fig. 10G). By the *ca.* 128-cell stage the blastomeres were closely spaced and columnar in shape (Fig. 10H). Ciliation was first seen at this time. Until now the embryos had remained on the bottom of the culture dishes. As ciliation increased, and with the advent of elongation and gastrulation the embryos began to swim and congregate at the surface of the dishes. At this stage, 6 to 8 hr AF, they became strongly attracted to light.

It is not clear by what process gastrulation took place. However there are some indications from the photomicrographs of the living embryos that gastrulation took place by primary delamination or mixed delamination (Mergner, 1971). The confirmation of this suggestion will have to await electron microscopy of the fixed embryos. No evidence of invagination or archenteron formation was seen in any of the embryos observed.

Within 12 to 15 hr AF the elongated gastrulae developed oral pores and apical ciliary tufts (planula stage) (Fig. 10 I). The young planulae swam and spun on their longitudinal axes quite rapidly, but still remained near the surface of the water. Over the next 24 hours the mouth and pharynx formed and the larvae became more elongated. From 48 to 72 hr AF the planulae became demersal or swam in mid-water; they no longer congregated at the surface, nor were attracted to light. The first signs of mesenterial development were noticed in a few of the larvae.

The embryos gradually decreased in size as development progressed. By the time the larvae reached the planula stage they were about 75 μm long—only $\frac{3}{4}$ to $\frac{1}{2}$ of the diameter of the original egg. Some of the culture dishes had an abundance of small flagellate, diatom and bacterial contaminants; but the planulae were never observed feeding on this material. Planulae in both "clean" and "contaminated" culture dishes shrunk at about the same rate. Attempts at presenting them with a variety of substrates failed to induce settlement, and evenutally all of the larvae died.

Discussion

What little is known about coral reproduction indicates that patterns of gametogenesis and development are quite variable (Connell, 1973; Rinkevich and Loya, 1979a). Previous studies have concentrated on mostly hermaphroditic species that brood their embryos and release a small number of large planulae (over 1 mm long) which settle and metamorphose soon after being spawned (Boschma, 1929; Abe, 1937; Marshall and Stephenson, 1933; Harrigan, 1972; Lewis, 1974; Stimson, 1976; Rinkevich and Loya, 1979a, b). Occasionally observations were made that indicated some coral species did not conform to this pattern of reproduction, but the information was too incomplete for alternate patterns of reproduction to be affirmed. The present study shows that *Astrangia danae* follows a different reproductive pattern: it is a dioecious coral, with large numbers of small eggs (*ca.* 0.1 mm diam.) and with external development. It is not clear at this time how these two different reproductive patterns, and their variants, are distributed among the ranks of coral families and genera, although Rinkevich and Loya (1979a) suggest that it may be related to gonad location within the polyp

and polyp size. They suggest that small-polyped species have gonads that bulge into the gastric cavity, produce small numbers of small eggs and brood their larvae, while large-polyped species have gonads within the mesenteries and produce large numbers of large eggs which are spawned and fertilized externally. The available information for *A. danae* and for several species of Caribbean reef corals suggests that there is no universal relationship between polyp size, egg size, and mode of development. *A. danae* which has moderately sized polyps (3–5 mm in diameter) differs from the model (if classified as a large-polyped species) by producing a large number of small eggs. The genus *Favia* presents a more complicated picture. *F. doryensis* from the Great Barrier Reef (Marshall and Stephenson, 1933) and *F. favus* from the Red Sea (Rinkevich and Loya, 1979a) are large-polyped species that have mesenterially located gonads with many large eggs. Although planulation has not been observed for either species, it is well documented for the Caribbean species *Favia fragum* (Duerden, 1902; Lewis, 1974) which also has mesenterially located gonads, but with only one to five large ova per gonad (Duerden, 1902). Therefore, related species with similar polyp and egg size differ in the number of eggs produced and in the manner of development. Duerden (1902) also observed planulation by the large polyped species *Manicina areolata* and *Isophyllia radiata*. It is not surprising that many reproductive patterns occur among the scleractinian corals, in light of the extreme diversity of reproductive patterns found among the closely related sea anemones (Spaulding, 1974; Campbell, 1974; Chia, 1976).

As far as we know, this is the first report on gametogenesis and embryonic development of a coral species. There have been, however, several studies of this kind on sea anemones (Campbell, 1974; Clark and Dewel, 1974; Spaulding, 1974; Jennison, 1979), with which the observations on *Astrangia* can be compared.

The gametogenetic patterns observed are, as expected, similar to those reported for other anthozoans (Campbell, 1974; Jennison, 1979). The interstitial cells which give rise to the germ cells are of endodermal origin and migrate into the mesenterial mesoglea before starting to differentiate. The spermaries in *Astrangia* remain within the mesoglea throughout their development; however, it appears that as the oocytes become very numerous and large, they are pushed out of the mesoglea into the endodermal portion of the mesentery. Trophonemata, umbilical-like connections between the gastrovascular cavity and growing oocytes that have been found in various groups of anemones (Dunn, 1975; Jennison, 1979), were not seen in *A. danae*.

The mature *Astrangia* eggs are similar in size and appearance to those of the sea anemone *Metridium* (Clark and Dewel, 1974). The sperm, however, do not resemble in nuclear shape or mitochondrial position those reported for other anthozoan species (Campbell, 1974; Clark and Dewel, 1974; Hinsch and Clark, 1973; Hinsch, 1974). The lipid body associated with the single mitochondrion is much larger in *Astrangia* than those reportedly associated with the mitochondria of *Bunodosoma* and *Metridium* (Clark and Dewel, 1974).

Astrangia development differs from patterns observed for sea anemones with similarly small eggs (Spaulding, 1974; Campbell, 1974) in that the first cleavage always results in a two-cell stage, and gastrulation does not appear to occur by invagination. Further elucidation of the similarities and differences between *Astrangia* and other forms will have to await studies using both light and electron microscopy.

The reproductive cycle of *A. danae* in Narragansett Bay seems to be controlled by temperature, although control by food availability cannot be ruled out. *Astrangia* colonies maintained in the laboratory at 20–22° C and fed on a regular schedule were able to spawn year round. It should prove interesting to study the reproductive cycles of populations of *A. danae* living along the Gulf of Mexico and the coasts of Florida where water temperatures are relatively warm all year.

Astrangia danae is known to occur naturally both with and without zooxanthellae. It was of interest to determine in the course of this study whether in *A. danae* the zooxanthellae were transferred to succeeding generations through the eggs or larvae. In no section were zooxanthellae found inside of eggs, although the algae were often very abundant in the gonadal tissues. The absence of zooxanthellae in the eggs was confirmed with the observation of freshly spawned eggs from symbiotic female colonies. Attempts to infect planulae with algae extracted from adult colonies were unsuccessful. However, the failure of any of these planulae to settle under our maintenance conditions leaves unanswered the question of if and when the planulae take up zooxanthellae.

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Summary

1. The coral *Astrangia danae* is dioecious, with an early age of first reproduction.

2. In Narragansett Bay, this species exhibits an annual reproductive cycle with gametogenesis starting in March–April. Spawning occurs during August, and vestigial gametes are absorbed during the winter months.

3. Gametes originate from interstitial cells which differentiate in the mesenterial mesoglea.

4. Ova are 100–130-μm in diameter and sperm are 2–3-μm in length, excluding the tails. Egg production was estimated at up to 6000 eggs per polyp.

5. Fertilization and development are external; no fertilization membrane was seen even though a layer of cortical vesicles was present before fertilization.

6. Embryonic cell divisions are around 30 min apart and the larval planual stage is reached within 12–15 hr after fertilization. Larval settlement was not observed.

7. Ova from colonies containing zooxanthellae did not contain zooxanthellae when spawned.

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